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THE EFFECT OF ALTITUDE AND CLOSED CONE (SEED) AGE ON GERMINATION IN RED PINE (*Pinus brutia* TEN.)

UTJECAJ NADMORSKE VISINE I STAROSTI ZATVORENOG ČEŠERA NA KLIJANJE SJEMENA CRVENOG BORA (*Pinus brutia* Ten.)

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SUMMARY

This study investigated changes in the germination rate and germination percentage of seeds obtained from closed pine cones (*Pinus brutia* Ten.) of different ages collected at different altitudes. The seeds used in the study were obtained from closed cones (3/control, 4, 5, 6, and 7 years old) in the lower (0-200 m) and higher (800-1000 m) altitude zones of the Mersin-Anamur region in a section from the sea to the interior. The seeds germinated in the dark at a constant temperature of 20 °C for 28 days. At the end of the germination test, a two-way analysis of variance was performed on the germination percentage data on the 10th, 14th, and 28th days, and the interactions between altitude and cone age were found to be significant ($p = 0.05$). As a result of the significant interaction between the two groups, the mean separation test (Tukey test) showed that the highest germination rate was 89.0% in the control group, and the lowest was 77.5% in the 7-year-old cones. In the higher altitude zone, the highest germination rate was 74.0% and the lowest was 71.0%, obtained from 5- and 7-year-old seeds, respectively. In general, germination values in the lower altitude zone were higher than those in the higher altitude zone. However, the decrease in germination values from the control to the 7-year-old seeds was greater in the lower altitude zone than in the higher altitude zone. The study found that altitude was more effective than cone age on the germination rate and percentage.

KEY WORDS: Red pine, closed cone, germination rate, germination percentage

INTRODUCTION
UVOD

Red pine (*Pinus brutia* Ten.) is distributed in and around the Mediterranean Basin, Palestine, Jordan, Syria, Iraq, Lebanon, Cyprus, Iran, Azerbaijan, Crimea, Greece, and Italy (Yaltırık 1993; Yıldız et al. 2004; Boydak et al. 2006; Yıldız et al. 2010). This species has the widest distribution in the world, with an area of 5.61 million hectares in Turkey, constituting one-fourth of Turkey's forest areas (Anonymous 2015). In the Mediterranean Region, red pine is generally found at altitudes ranging up to 1300 m and in some places as high as 1500 m. This species has high genetic diversity, adapts easily to different ecological conditions, and grows rapidly (Boydak et al. 2006; Yıldız et al. 2010; Yıldız et al. 2018) Therefore, because this species establishes forests in a wide-ranging geography under different conditions and grows rapidly, forestry wood and non-wood ecosystem services are quite high in Turkey. The intra-population variation in red pine seed germination rates and percentages is quite high (Thanos 2000; Boydak 2004; Boydak et al. 2006). Previous studies show that the temperature at which red pine seeds germinate is generally 15-20 °C (Şefik

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1965; Thanos 2000). Çetin (2010) germinated the seeds collected from different altitudes in the Mediterranean Region at different temperatures and the highest germination was achieved at 20 °C.

Differences in germination rates can be observed depending on the altitude at where the seeds are collected (Şefik 1965; Ürgenç 1977; Çetin 2010). Red pine cones generally mature in May, and the cones are opened and the seeds dispersed until August. However, some cones can remain on the tree for a long time without opening (Selik 1963; Şefik 1965; Thanos and Doussi 2000).

In particular, the importance of preserving the germination feature of red pine cone seeds emerges when they are in areas of fire-susceptible ecosystems. It is said to be effective in the re-formation of pine forests when these closed cones open after a fire and the seeds fall to the ground and germinate after the first rains (Boydak et al. 2006). Fernandes et al. (2019) determined that in general, serotinous cones make a significant contribution to the regeneration of *Pinus pinaster* and *Pinus halepensis* forests after a fire. Pine species may differ in terms of forming closed cones (Feduck et al. 2015). In addition, according to the ecosystems where they are found, varieties of the same pine species can differ in terms of the age and amount of closed cones. For example, Çetin (2010), in his study on the species, found that the lower altitude zone produced more cones and older cones than the higher altitude zone. He emphasized that serotinous cone level, tree age, and fire severity are important in a *Pinus pinaster* forest. The seeds obtained from these serotinous cones were determined to have a higher viability and germination percentage than those of non-serotinous cones (Cruz et al. 2019).

At the end of the germination period, a high rate of seed germination in a short time is just as important as a high percentage of seed germination. The success of planting and natural rejuvenation depends on the germination rate (speed) rather than the germination percentage (Saatçioğlu 1971). One seed with a high germination rate (i.e., with a steeper germination graph on the 7th, 10th, and 14th days) is more valuable in creating seedlings than two seeds with the same germination percentage (Saatçioğlu 1971). Consequently, seeds with a good germination rate are more beneficial in planting, natural rejuvenation, and rejuvenation occurring spontaneously after a fire because they germinate in a short time and have a good chance of developing and establishing a forest. In forests such as red pine that are constantly exposed to fire, it is extremely important to preserve the germination feature and contribute to the formation of progeny in the seeds of the older cones of other years, as well as in the normal three-year mature cones.

No previous study has investigated the genetic characteristics of the serotinous status of red pine. However, one study determined that *Pinus halepensis* had a significantly narrow serotinous status heritability (Hernandez-Serrano et al. 2014). In addition, *Pinus concorta* was mentioned as having gene diversity in serotiny, debatably under the influence of many genes rather than one or two genes (Parchman et al. 2012). In the areas of the closed cone-forming variety, the seeds preserved their germination feature and contributed to regeneration after fires (Su et al. 2015). In red pine, not only the seeds of the past year, but also the seeds of other years will join the progeny, and this progeny consisting of seeds of different years will have the opportunity to develop more forests that exhibit genetic diversity.

Finally, studies have shown that there may be differences in the germination rate of red pine seeds depending on the age of the cones and the altitude at where the cones are collected. This study aimed to determine the germination rate at 10 and 14 days and the total germination rate at the end of 28 days of seeds obtained from closed cones of five different ages (3/control, 4, 5, 6, and 7 years) collected at two different altitudes (0-200 and 800-1000 m).

MATERIAL AND METHODS
MATERIJALI I METODE

Sampling sites – *Mjesta uzorkovanja*

Seeds were collected from 20-25-year-old red pine stands, naturally distributed on the southern slopes of the Taurus Mountains, within the borders of the Anamur Forest Enterprise of the Mersin Regional Directorate of Forestry. The aged cones were selected from low-altitude (0-200 m) red pine stands in the Anamur Forest Chiefdom (N:36° 02’ 10”, E:32° 42’ 25”- UTM ED50 36N) and from the high-altitude (800-1000 m) forests of the Sarıdana Forest Chiefdom (N:36° 10’ 11”, E:32° 41’ 34”). Closed cones aged 3, 4, 5, 6, and 7 years were collected in summer (July) from trees at both elevation levels.

METHOD
METODA

The collected cones were laid on a flat surface and in order to open them faster, they were sprayed and mixed with water under the sun, and they subsequently opened in a short time. The wings were separated from the winged seeds in the cone manually and by machine, and the cleaned seeds were placed in jars and stored in the refrigerator (1-4 °C) until the germination tests. Germination experiments were carried out at 20 °C ambient temperature in 9-cm-diameter glass Petri dishes, with seeds placed on filter paper in such a way that they did not touch each other. Trial units (2 × 5 = 10) were created for the seeds obtained from the cones collected at five different ages from both elevation levels.

Table 1. Germination percentages and standard deviation of seeds extracted from different-aged red pine cones from low and high altitude zones on the 10th, 14th, and 28th days
Tablica 1. Postoci klijavosti i standardna devijacija sjemena ekstrahiranog iz češera crvenog bora različite starosti iz zona niske i visoke nadmorske visine 10., 14. i 28. dana

Altitude/Elevation zone (m) Nadmorska visina/visinska zona (m)	Cone age (years) **Starost** češera (godine)	Germination percentage (%) Postotak klijavosti (%)		
		10th day 10. dan	14th day 14. dan	28th day 28. dan
0-200 m	3	20.5±2.2a*	62.5±6.0a	89.0±1.7a
	4	13.5±1.7ba	58.0±1.0ba	87.0±1.3ba
	5	10.5±1.0b	56.0±2.4ba	85.5±1.3ba
	6	12.5±2.5ba	51.0±1.7bac	81.0±1.0bc
	7	11.5±3.0ba	46.5±4.3bdc	77.5±2.4dc
800-1000 m	3	15.5±2.7ba	43.5±4.7bdc	74.0±1.2de
	4	11.0±1.3ba	34.0±2.2d	72.0±1.2de
	5	10.0±2.6b	39.0±1.9dc	71.0±1.0e
	6	9.5±1.3b	39.0±1.7dc	72.0±0.8de
	7	6.5±1.9b	33.0±1.3d	71.0±1.3e

*Means within the same column with different letters are significantly different ($p \leq 0.05$)
*Srednje vrijednosti unutar istog stupca s različitim slovima značajno se razlikuju ($p \leq 0,05$)

Each trial unit used 50 seeds and trials were repeated four times. Therefore, a total of 10 trial units × 50 seeds × 4 replicates = 2000 seeds tested. During the 28 days, i.e., the total germination period, the seeds were monitored daily for deterioration and fungal growth, and distilled water was used to meet the water requirement of the seeds. In the experiment, the seeds that had rootlets extending to the size of the seed were considered germinated. The germination rate indicates the ability of seeds to germinate quickly. The germination rate of the germinated seeds was recorded on the 7th, 10th, and 14th days (Saatçioğlu 1971). However, if some types of seeds such as red pine are not subjected to pre-treatment such as stratification, etc., germination may not occur in seven days (Çetin 2010). In this study, since germination started after seven days, the germination rate was calculated from the germination data on the 10th and 14th days, and the overall germination percentage at the end of the 28th day. Germination rate and germination percentage were calculated from the seeds germinated using the following formula:

$$GP\ (\%) = \frac{\sum ni}{N} x100$$

GP (%): Germination percentage
ni: The number of germinated seeds on day i
N: The total number of tested seeds

Statistical analyses – *Statističke analize*

A two-way interaction test was run for the seed germination rates for three periods (10, 14, and 28 days). Altitude zones were at two elevation levels (L=low and H= high) and the cone age at five levels (3, 4, 5, 6, and 7 years). Since the interactions between altitude zone and cone age were significant on seed germination for all the tree periods (p = 0.005), the cone-age data were merged with data of the corresponding altitude zones and the Tukey mean separation analysis was conducted on the combined data. The SAS (1996) package program was used in the analysis. The results were considered different at the $\alpha = 0.05$ level.

RESULTS
REZULTATI

The first germination in the seeds was on the 8th day. As seen in Table 1, the fastest and highest germination among all sample areas was realized in the seeds obtained from the 3-year-old (control) cones in the 0-200 m lower altitude zone. Germination at 10, 14, and 28 days was 20.5%, 62.5%, and 89.1%, respectively. The difference in the germination rate between seeds of different ages gradually decreased towards the end of the germination period (28 days) (Table 1; Figs 1, 2, and 3).

Seed germination rates of less than 20% were recorded for all cone types in the first 10-day period of the experiment. In this period, the highest germination was in L3 and this value was 95% higher than L5. The germination rate in the lower altitude zone was higher than that in the higher altitude zone. The lowest value in the entire trial was in H7, which was about half of the average germination rate of both altitude zones (Fig. 1).

Germination rates had increased 2 - 3 times by the 14th day of the experiment. In this period, the germination rate of L3 was 34% higher than that of L7, and L3 had also a 67% higher germination rate compared to that of the average of the

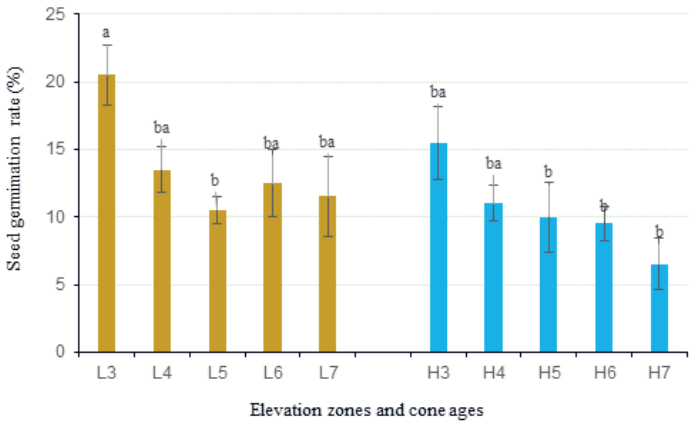

Figure 1. Means ± std. err. for germination rates (10 days) of seeds obtained from varying aged (3, 4, 5, 6, and 7 years) cones collected at low (L = 0-200 m) and high (H = 800-1000 m) altitude zones. Means followed with the same lower-case letters are not significantly different at $\alpha = 0.05$.

Slika 1. Srednje vrijednosti ± std. pogreške za stope klijanja (10 dana) sjemena dobivenog iz češera različite starosti (3, 4, 5, 6 i 7 godina) sakupljenih u zonama niske (L = 0-200 m) i visoke (H = 800-1000 m) nadmorske visine. Srednje vrijednosti praćene istim malim slovima ne razlikuju se značajno pri $\alpha = 0,05$.

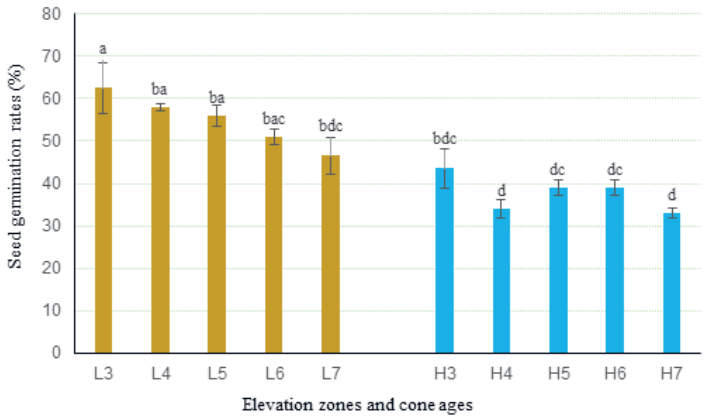

Figure 2. Means ± std. err. for germination rates (14 days) of seeds obtained from varying aged (3, 4, 5, 6, and 7 years) closed cones collected at low (L= 0-200 m) and high (H= 800-1000 m) altitude zones. Means followed with the same lower-case letters are not significantly different at $\alpha = 0.05$.

Slika 2. Srednje vrijednosti ± std. pogreške za stope klijanja (14 dana) sjemena dobivenog iz zatvorenih češera različite starosti (3, 4, 5, 6 i 7 godina) sakupljenih na niskim (L= 0-200 m) i visokim (H= 800-1000 m) nadmorskim visinama zonama. Srednje vrijednosti praćene istim malim slovima ne razlikuju se značajno pri $\alpha = 0,05$.

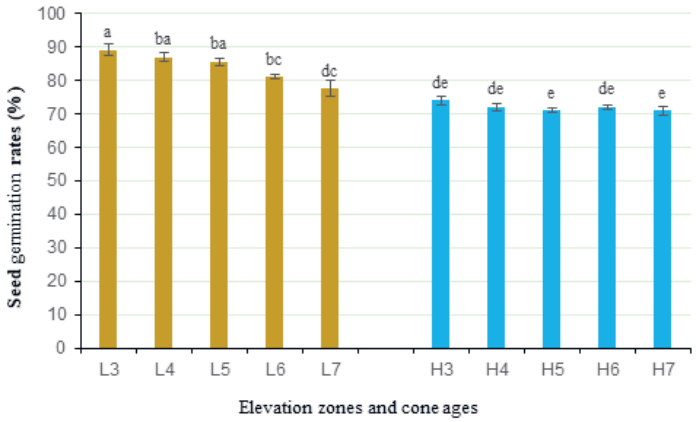

Figure 3. Means ± std. err. for germination rates (28 days) of seeds obtained from varying aged (3, 4, 5, 6, and 7) closed cones collected at low (L = 0-200 m) and high (H= 800-1000 m) altitude zones. Means followed with the same lower-case letters are not significantly different at $\alpha = 0.05$.

Slika 3. Srednje vrijednosti ± std. pogreške za stope klijanja (28 dana) sjemena dobivenog iz zatvorenih češera različite starosti (3, 4, 5, 6 i 7) sakupljenih u zonama niske (L= 0-200 m) i visoke (H= 800-1000 m) nadmorske visine. Srednje vrijednosti praćene istim malim slovima ne razlikuju se značajno pri $\alpha = 0,05$.

higher altitude zones In the higher zone, H3 had germinated approximately 25% more than H7. However, the difference in the germination rate between cone ages in both altitude zones had decreased compared to the 10th day (Fig. 2).

More than 70% of the seeds had germinated for each cone type by the 28th day of the experiment. As the germination period progressed and at the end of the germination period, the difference in the germination rate between cone ages decreased. The lowest germination rates were recorded for H5 and H7 cone types, and L3 and L4 had about 19% higher germination rates compared to the average of cones collected at the higher altitude zone (Fig. 3).

DISCUSSION
RASPRAVA

Like many pine species distributed in the Mediterranean Basin, which is susceptible to fire, red pine also forms a large number of cones, and some of these cones remain on the crown for several years without opening (Verkaik and Espelta 2006). The seeds in the cones that remain closed on the tree can maintain their germination properties for a certain period (Selik 1963; Şefik 1965; Boydak 2004). In fire-susceptible Mediterranean ecosystems, serotinous cone structure is seen as an assurance for the continuation of the red pine species (Neyişci and Cengiz 1985; Cengiz 1993, Thanos and Doussi 2000; Boydak et al. 2006). Therefore, the seeds kept in these closed cones over a long time make an important contribution to the seed pool for the generation to come in the region after destruction, e.g., a fire event (Fernandes et al. 2019).

In a study on the detection of closed cones in red pine, Çetin (2010) determined that more cones remained closed for a long time in trees at low altitudes (0-250 m). Selik (1963) obtained close germination percentages of 53.7% and 51%, respectively, from seeds of three- and nine-year-old closed red pine cones. In addition, Şefik (1965) germinated seeds obtained from three – six-year-old closed cones and determined that the effect of cone age on the germination capacity of the seeds was very small. Ürgenç and Odabaşı (1971) found that when mature cones were collected from trees and stored for seven years at room temperature without opening, they germinated, and although there was a slight decrease in germination, their germination rate was above 50%. However, there was no difference between the germination of seeds stored at low temperatures and those of closed cones kept at room temperature. The germination characteristics of the seeds are also determinant in the intra-species competition of the future generations in the region and affect the dynamics of the stand at the establishment stage. Germination percentage is widely used to determine the germination characteristics of seeds. The ability of the seed to germinate quickly, i.e., the germination rate, is an especially important issue. The germination rate also determines the intra-species competition of the future generations in the region. Saplings formed by fast-germinating seeds adapt better to environmental growing conditions (Saatçioğlu 1971; Dağlar et al. 2016). Germination rates and percentages affect the dynamics of the forest in the establishment phase, especially in areas where there are long summer droughts (Dunlap and Barnet 1984).

In this study, the highest germination rate (20.5% on the 10th day) was observed in the seeds obtained from 3-year-old cones from the lower altitude zone. During this period, the germination rates of the seeds obtained from the 5-, 6-, and 7-year-old cones in the higher altitude zone remained below 10%. However, on the 14th day of the experiment, the germination rate of the seeds obtained from 3-year-old cones in the lower altitude zone increased to 62.5%. In their study on the species, Şefik (1965) and Çetin (2010) determined that the germination rate of seeds could be increased significantly by stratification processes. An important factor affecting germination is the elevation level at which the seeds are collected (Çetin 2010). Eler (1992) found no missing seeds in closed cones of different ages collected from three different altitude zones (0-400 m, 400-800 m, and above 800 m), and germination rates of 90%, 81%, and 60% were obtained from cones of three consecutive years. Çetin (2010) determined that the germination rate of seeds collected from lower (0-400 m) and middle (400-800 m) elevations was higher than that of seeds collected from higher elevations (above 1200 m). This study determined that the elevation level where the cones were collected was more effective than the cone age in the germination rate values on the 14th day. The results of the study showed that the germination rate of seeds decreased as the altitude and age of the cones increased. The altitude zone where the seeds were collected was more effective in this decrease than the age of the cones. The results of previous studies as well as this study showed that although the germination rates of the seeds obtained from closed pine cones of increasing cone age differed according to their characteristics, they were able to preserve the germination feature at a certain rate. When the closed cones open after a fire and the viable seeds of the species scatter and suitable germination conditions are formed, important contributions are made to the future generations of red pine (Boydak et al. 2006).

Observations and some studies have shown that the viability of these seeds in the cones is generally not affected during a fire, since the serotinous cones are closed during fires. These cones, which are exposed to high heat during a fire, open slowly after the fire and the seeds fall into the soil. When these fallen seeds find a good germination environment in the ashy fire area and reach the appropriate germination temperature after the rains, they contribute to the new germinating generation. Obvious examples of this were seen in our observations at major fire sites in Antalya, Mersin, and Muğla in July 2021.

Many studies have determined that, as with red pine, some other pine species produce closed cones and that these cones are opened after a fire. For example, it is stated that the closed cones of *Pinus pinaster*, which is distributed in fire-prone ecosystems, make an important contribution to future generations by opening and germinating after a fire (Moya et al. 2018). Again, a study conducted with *Pinus yunnanensis* pine species in 80 sample areas in China found that closed cones were formed in all of the regions where the species grew, but the percentage of closed cones decreased as the age of the closed cones increased. The germination rate of 1- and 2-year-old closed cones in these regions was around 90%. As the age of the closed cones increased, the germination rate of the seeds decreased, with the germination rate of 7-year-old seeds decreasing to approximately 32%. It was determined that the lower-aged closed cones in this region opened and germinated after a fire and played an important role in the regeneration of *Pinus yunnanensis* forests (Su et al. 2015). The amount of serotinous cones is effective on tree age and silvicultural interventions such as thinning. The ability of the seeds in these cones to maintain their germination feature also depends on the severity and duration of the fire. It was determined that especially after severe fires, the seeds in the closed cones lost their viability and thus, the number of *Pinus pinaster* and *Pinus halepensis* saplings decreased. After less severe forest fires, it was determined that the seeds in the closed cones germinated and made an important contribution to the regeneration of the forests. Fernandes et al. (2019), in another study on *Pinus halepensis*, reported that the number of closed cones might vary according to the age of the stand and the silvicultural interventions applied and that they could be effective in the formation of a new generation after a fire (Verkaik and Espelta, 2006).

No previous study has examined the genetic characteristics of the serotiny status of red pine. However, in a study conducted to determine the heritability of the serotiny status of *Pinus halepensis*, which like red pine has serotinous properties, the serotiny status showed a significantly narrow heritability of 0.20. Although this species exhibited a high variability within the serotinous population, forests with repeated wildfires contained more serotinous species than those rarely exposed to fire (Hernandez-Serrano et al. 2014). This view is supported by observing that the ecosystems in the Mediterranean Basin where red pine forests showing sertinous features are distributed have been constantly exposed to fire in the past. Although no study has examined the genetic characteristics of the species in terms of serotinous features, this subject has been considered in other pines. For example, in a study carried out to determine the genetic basis of the serotinous cones of *Pinus concorta*, differences in serotinous characteristics were found in some trial areas, and different genes and ratios were obtained from serotinous cones and non-serotinous cones in some trial areas (Feduck et al. 2015). Thus, although the germination rates and percentages in serotinous cones were slightly lower than for 3-year-old cones, considering that these cones may have different genetic characteristics, the use of the seeds in these cones could have a positive effect on the genetic diversity of red pine forests and the sustainability of forests and their adaptation to global climate change.

CONCLUSIONS
ZAKLJUČAK

In this study, although the germination rate of seeds obtained from closed cones 3 - 7 years of age was low on the 10th day, more than 50% of the seeds obtained from the lower altitude zone and approximately 40% of the seeds from the higher altitude zone had germinated on the 14th day. Although the germination rates differed among the cone ages at the beginning, this difference in germination values decreased as time progressed. It was determined that the germination rate of the seeds obtained from the 7-year-old closed cones collected at both altitudes was still above 70%. As a result, when these aged cones open after a fire, the seeds dispersed will contribute extensively to re-establishing the forest in burned areas left without any rejuvenation work after the fire, and in steeply sloping areas and in places where it is not possible to establish a forest by human hand. Considering that the closed serotinous cones of other pine species exhibit additional genetic characteristics (Feduck et al. 2015), the use of seeds from these cones to grow saplings and establish forests may provide an advantage in terms of increasing genetic diversity.

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SAŽETAK

U ovom istraživanju analizirane su promjene u stopi klijavosti i postotku klijavosti sjemena iz zatvorenih češera *Pinus brutia* Ten. različite starosti, sakupljenih na različitim nadmorskim visinama. Sjeme korišteno u istraživanju dobiveno je iz zatvorenih češera (3/kontrola, 4, 5, 6 i 7 godina starosti) u nižim (0-200 m) i višim (800-1000 m) visinskim zonama Mersin- Anamurske regije rasprostranjenih od mora prema unutrašnjosti. Sjeme je klijalo u mraku pri konstantnoj temperaturi od 20 °C 28 dana. Na kraju testa klijavosti provedena je dvostruka analiza varijance o postotku klijavosti 10., 14. i 28. dana, te je utvrđeno da su interakcije između nadmorske visine i starosti češera značajne ($p = 0,05$). Kao rezultat značajne interakcije između dviju skupina, srednji test razdvajanja (Tukey test) pokazao je da je najveća klijavost bila 89,0% u kontrolnoj skupini, a najniža 77,5% u 7-godišnjim češerima. U zoni viših nadmorskih visina najveća klijavost iznosila je 74,0%, a najmanja 71,0%. Dobivena je iz sjemena starih 5 odnosno 7 godina. Općenito, vrijednosti klijavosti u zoni niže nadmorske visine bile su veće od onih u zoni više nadmorske visine. Međutim, smanjenje vrijednosti klijavosti od kontrolnog do 7-godišnjeg sjemena bilo je veće u zoni niže nadmorske visine nego u zoni više nadmorske visine. Studija je pokazala da je na stopu klijanja i postotak, nadmorska visina učinkovitija od starosti češera.

KLJUČNE RIJEČI: crveni bor, zatvoreni češer, klijavost, postotak klijavosti